Biochem. J. (1978) **169**, 617–624
Printed in Great Britain

The Isolation and Properties of a Non-Pepsin Proteinase from Human Gastric Mucosa

By NORMAN B. ROBERTS and WILLIAM H. TAYLOR
Department of Chemical Pathology, Liverpool Area Health Authority (Teaching), Central Southern District, Ashton Street, Liverpool L3 5RT, U.K.

(*Received* 14 *October* 1977)

1. A non-pepsin proteinase, proteinase 2, was successfully isolated free from pepsinogen (by repetitive chromatography on DEAE- and CM-celluloses) from the gastric mucosa of a patient with a duodenal ulcer and the uninvaded mucosa of a patient with a gastric adenocarcinoma. 2. Proteinases 1a and 1b, found in gastric adenocarcinoma, were not found in the gastric mucosa of these patients. 3. Proteinase 2 was shown to have an asymmetrical broad pH–activity curve with a maximum over the pH range 3.0–3.7. 4. Proteolytic activity of proteinase 2 was inhibited by pepstatin; the concentration of pepstatin giving 50% inhibition is of the order of 3 nM. 5. Inhibition of proteolytic activity by carbenoxolone and related triterpenoids indicated that at pH4.0 proteinase 2 possesses structural characteristics relating it to the pepsins and at pH7.4 to the pepsinogens. 6. The sites of cleavage of the B-chain of oxidized insulin for proteinase 2 at pH1.7 and pH3.5 were shown to be similar to those previously established for human pepsin 3 and for the cathepsin E of rabbit bone marrow. 7. The non-pepsin proteinase 2 (cathepsin) of human gastric mucosa has properties more similar to cathepsin E than to the cathepsins D.

Proteolytic activity of acidified human gastric-mucosal extracts on agar-gel electrophoresis at pH5.0 revealed up to eight individual zones (Etherington & Taylor, 1970). Those enzymes numbered 1, 2, 3, 3a and 5 represent individual pepsins; zones 4 and 6, when they occur, represent a pepsin–pepsin-inhibitor complex and unchanged pepsinogen respectively. All these zones can be destroyed by adjustment to pH8.0 and are therefore classified as pepsins. Zone 7 is not so destroyed and is thus a non-pepsin proteinase.

Pepsins are absent from extracts of human gastric adenocarcinomata (Etherington & Taylor, 1972), but a zone 7, non-pepsin proteinase, is present. Agar-gel electrophoresis of acidified extracts of human gastric adenocarcinomata at pH8.2 resolved this non-pepsin zone into four components, comprising two anodal zones (proteinases 2a and 2b) and two cathodal zones (proteinases 1a and 1b). Etherington & Taylor (1972) also showed that only proteinases 2a and 2b were present in normal human gastric mucosa.

It was decided, therefore, to study further the non-pepsin proteinase 2 (zone 7) of human gastric mucosa, which hitherto had not been characterized or isolated, and compare its properties with those of the proteinases from human gastric adenocarcinomata, as reported by Etherington & Taylor (1972).

Experimental

Materials

Human gastric-mucosal extracts were prepared from specimens obtained after surgical operations, as previously described by Etherington & Taylor (1969). One patient had a duodenal ulcer and the second a gastric carcinoma. In the latter instance only the uninvaded mucosa was used. Crystalline pig pepsin and pepsinogen were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.; bovine haemoglobin substrate powder was from Armour Laboratories, Eastbourne, Sussex, U.K. Pepstatin (sodium salt) was kindly given by Dr. H. Umezawa and Dr. K. Goto of the Banyu Pharmaceutical Co., Tokyo, Japan. Carbenoxolone (disodium salt) and the derivatives of glycyrrhetic acid were kindly given by Biorex Laboratories, London N1 2HB, U.K. Bovine insulin (six-times recrystallized), obtained from Boots, Nottingham, Notts., U.K., was oxidized and the B-chain separated out as described by Sanger (1949). DEAE-cellulose DE-52 and CM-cellulose CM-11 were obtained from Whatman Biochemicals, Maidstone, Kent, U.K.

Methods

Agar-gel electrophoresis. The gastric extracts and

chromatographic fractions were analysed on agar-gel electrophoresis at pH5.0 (Etherington & Taylor, 1969) and at pH8.2 (Lapresle & Webb, 1962). In this technique, the proteinases migrate at different speeds towards the anode; after electrophoresis, the agar plates are immersed in human globin solution (Ito *et al.*, 1964) for 30min, then incubated at 37°C for 30min to allow proteolysis to occur. The plates are then fixed and stained with Ponceau S; the areas of proteolysis show up as translucent zones. To test for the existence of acid-activated zymogens, extracts were acidified to pH2.0 and incubated at 37°C for 10min before application to the gels.

Ion-exchange chromatography. Separation of the non-pepsin proteinase 2 by ion-exchange chromatography was carried out by a combination of the methods reported by Etherington & Taylor (1969, 1972). Any alteration in gradients applied are given in the legends to the appropriate Figures.

Determination of proteolytic activity. The proteolytic activity was determined by the method of Anson & Mirsky (1933) as modified for cathepsins by Etherington & Taylor (1972). Incubation was carried out at 37°C in 0.2M-sodium acetate adjusted with 0.2M-HCl to pH3.7 and containing 3.3g of bovine haemoglobin/litre. Proteolytic activity is expressed as A_{700}

Construction of pH–activity curve. Solutions of 0.2M-sodium acetate and 0.2M-HCl, each containing 3.3g of bovine haemoglobin/litre, were mixed to give a range of solutions from pH1.5 to pH5.5, and the proteolytic activity was determined, as outlined above. Blank readings were taken at each pH. pH was determined with a Vibron pH-meter, model 39A (E.I.L., Richmond, Surrey, U.K.).

Inhibition of peptic activity. Pepstatin was prepared as an homogenized stock suspension of concentration 70.6μM in 0.05M-sodium acetate/acetic acid buffer at pH4.0. Serial dilutions of the stock solution were then made with the pH4.0 buffer. To 0.05ml of an appropriate pepstatin solution was added 1.9ml of the pH3.7 proteolytic assay solution. The solutions were equilibrated at 37°C for 5–10min, and 0.05ml of the enzyme solution in 0.001M-HCl was then added to start the reaction. The concentration of pepstatin referred to in the text is that in the final mixture.

For experiments with carbenoxolone and the glycyrrhetic derivatives, inhibition studies were carried out as described by Roberts & Taylor (1973) and Waft *et al.* (1974).

Digestion of the B-chain of oxidized insulin. The method used for the digestion of the B-chain of oxidized insulin was that of Sanger & Tuppy (1951) as modified by Etherington & Taylor (1971, 1972). The incubation solutions were adjusted to pH3.5 and pH1.7 with 1M-HCl. Incubation was then carried out for 5h and 24h at 37°C.

Calculations

The amount of proteinase activity was expressed

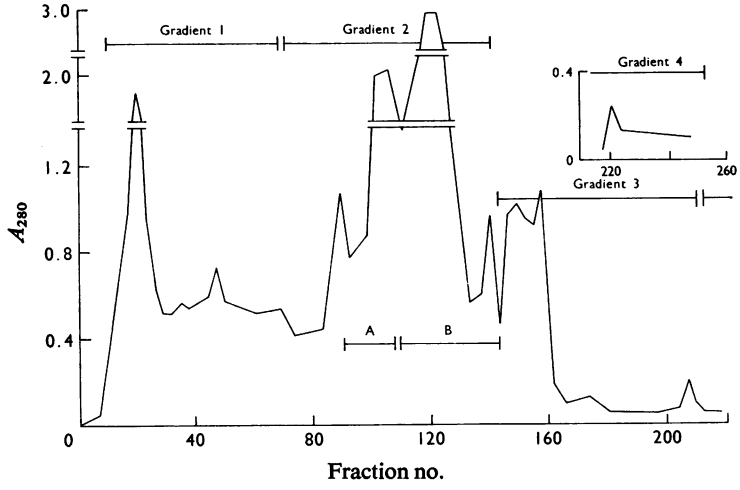

Fig. 1. *DEAE-cellulose chromatography at pH8.0 of a human fundic-mucosal extract*

Linear gradients used were: 1, 400ml of 0.005M-potassium phosphate buffer, pH8.0,+400ml of 0.05M-potassium phosphate buffer, pH8.0. 2, 400ml of 0.05M-potassium phosphate buffer, pH8.0,+400ml of 0.05M-potassium phosphate buffer (pH8.0)/0.5M-NaCl. 3, 400ml of 0.05M-potassium phosphate buffer (pH8.0)/0.5M-NaCl+400ml of 0.05M-potassium phosphate buffer (pH8.0)/1.0M-NaCl. 4, 300ml of 0.05M-potassium phosphate buffer (pH8.0)/1.0M-NaCl+300ml of 0.05M-citric acid. Fraction size was 11ml. ——, Protein (A_{280}). Fractions indicated by A contained proteinase 2 and non-enzymic protein, and those indicated by B contained pepsinogens, proteinase 2 and non-enzymic protein.

in terms of the equivalent activity of crystalline pig pepsin A. Proteinases were buffered at pH3.7 and compared with known pepsin A concentrations that had been incubated at pH1.9.

Where standard parametric correlation coefficients were calculated, the data were distributed sufficiently closely to bivariate normality for the *P* values to be calculated.

Results

Isolation of proteinase 2

Fig. 1 shows the protein-elution profile of a human fundic-mucosal extract after DEAE-cellulose chromatography at pH8.0. Analysis on agar-gel electrophoresis at pH5.0 and pH8.2 (Fig. 2) showed that fractions up to 90 had no proteolytic activity;

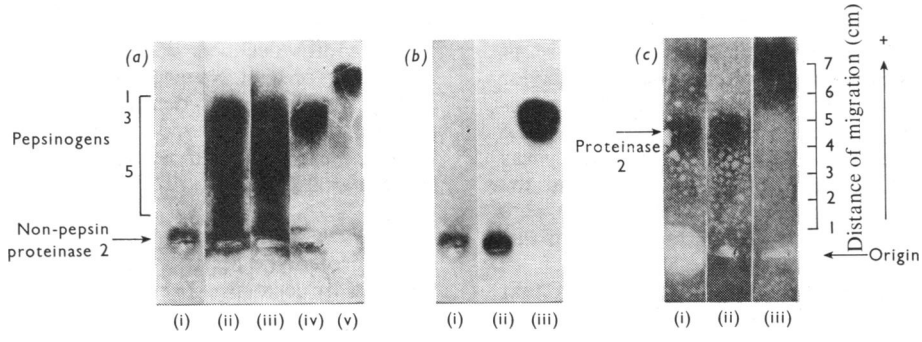

Fig. 2. *Analysis of pooled fractions from Fig.* 1 *on agar gel*

Electrophoresis was carried out for 3h at 2mA/cm width and 11V/cm length at pH5.0 and at pH8.2. (*a*) Fractions 91–110 (i), 111–127 (ii) and 128–142 (iii) eluted at 0.10–0.20M-Cl^-, 0.20–0.35M-Cl^- and 0.35–0.50M-Cl^- respectively. Pig pepsinogen (iv) and pepsin (v) were run as markers. pH5.0 electrophoresis. (*b*) Comparison of the mobility of the zone of activity remaining at the origin (fractions 91–110) before (i) and after (ii) acid activation. Pig pepsin (iii) was run as marker. pH5.0 electrophoresis. (*c*) Fractions 91–100 (i) and 101–110 (ii) after electrophoresis at pH8.2. Pig pepsinogen (iii) was run as a marker; 1 μg of each enzyme marker was applied to the gels.

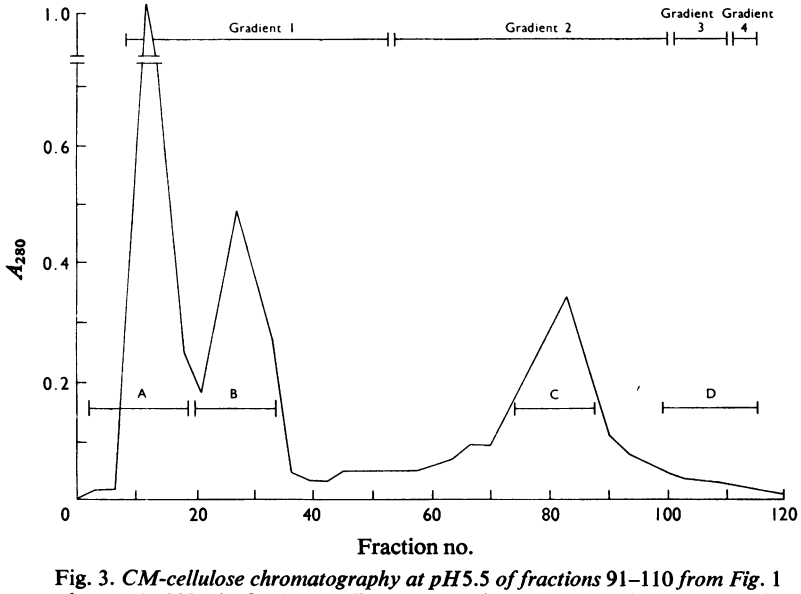

Fig. 3. *CM-cellulose chromatography at pH5.5 of fractions* 91–110 *from Fig.* 1

Linear gradients used were 1, 300ml of 0.01M-sodium acetate (pH5.5)+300ml of 0.05M-sodium acetate (pH5.5). 2, 300ml of 0.05M-sodium acetate (pH5.5)+300ml of 0.05M-sodium acetate (pH5.5)/0.5M-NaCl. To ensure that all the proteolytic activity was eluted, 40ml of 0.05M-acetate buffer, pH5.5, containing 1.0M-NaCl (gradient 3) and then 80ml of 0.05M-potassium phosphate buffer, pH7.4 (gradient 4), were washed through the column. Fraction size was 11ml. ——, Protein (A_{280}). A, proteinase 2+non-enzymic protein; B, C and D, non-enzymic protein.

fractions 91–110 contained only the non-pepsin proteolytic activity, and it should be noted that after acidification the mobility of this zone did not change and that, on electrophoresis at pH 8.2 (Fig. 2c), proteinase 1 was not found; fractions 111–140 that contained proteolytic activity contained mixtures of pepsinogens together with some non-pepsin proteinases. Elution of the non-pepsin enzyme occurred with the second gradient over the range 0.10–0.20 M-NaCl in 0.05 M-potassium phosphate buffer, pH 8.0.

The fractions containing the non-pepsin proteinase were then chromatographed on CM-cellulose at pH 5.5, which was used by Etherington & Taylor (1972) to separate the proteinases 1. All the proteolytic activity was eluted in the early fractions of the first gradient (fractions 0–19, Fig. 3). No activity was detected with the subsequent gradient, so that the proteinases 1 of Etherington & Taylor (1972) were absent from the two mucosal extracts used in this study.

The protein-elution profile (Fig. 3) shows that chromatography on CM-cellulose separates off much non-enzyme protein. The fractions containing proteolytic activity thus obtained were further chromatographed on DEAE-cellulose at pH 5.3 in order to remove non-enzymic protein and for a second time on DEAE-cellulose at pH 8.0 in order to try to separate the proteinase-2 components. Fractions eluted over the range 0.1–0.2 M-NaCl showed a major zone of proteolytic activity, with a trace of activity in those eluted at 0.2–0.4 M (Fig. 4). Proteolytic activity was not detected in any other of the pooled fractions. Unfortunately, it was not certain from the agar-gel analysis whether the proteinase 2 had two components.

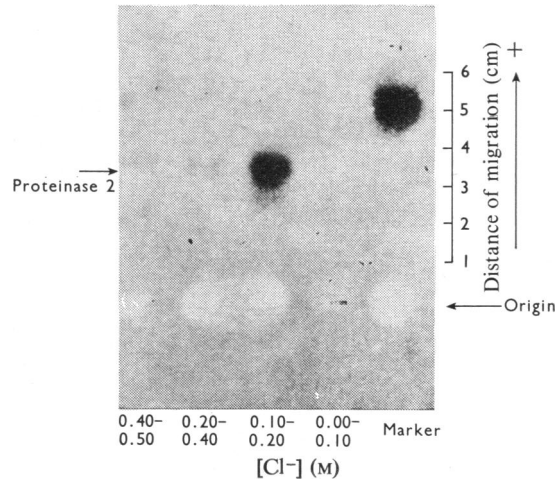

Fig. 4. *Agar-gel electrophoresis at pH 8.2 of fractions obtained with increasing Cl^- concentrations after chromatography of human gastric proteinase 2 on DEAE-cellulose at pH 8.0 with the use of gradients 1 and 2 shown in Fig. 1*
Electrophoresis was carried out for 3 h at 2 mA/cm width and 11 V/cm length. Pig pepsinogen was run as a marker (1 μg applied).

pH–activity curve

The pH–activity curve for the proteinase 2 is given in Fig. 5. From pH 1.5 proteolytic activity increased steadily to a plateau of fairly constant activity over the pH range 2.5–3.5. The activity beyond pH 3.5 then steadily declined until at pH 5.5 very little activity was present. No evidence of a sharp maximum point of activity was detected. The proteinase 2 pH–activity curve was very similar to that recorded for the proteinase 2 of adenocarcinomata extracts (Etherington & Taylor, 1972).

Effect of pepstatin

The proteolytic activity was shown to be readily inhibited by pepstatin (Table 1); the concentration of pepstatin giving 50% inhibition of enzyme activity was of the order of 3.0 nM. A similar value of 4.5 nM

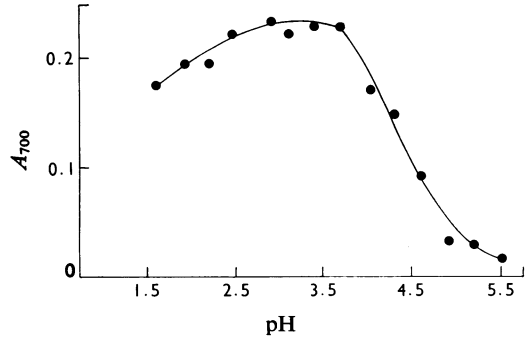

Fig. 5. *pH–activity curve for human gastric proteinase 2 with bovine haemoglobin as substrate*

Table 1. *Effect of increasing concentrations of pepstatin on the proteolytic activity of human gastric proteinase 2*
The amount of enzyme used was 0.6 μg expressed in pig pepsin equivalents. Incubation at 37°C was carried out for 30 min.

Concentration of pepstatin (nM)	Inhibition of proteinase 2 (% of initial activity remaining)
0	100
0.35	94
1.77	65
3.50	45
17.7	4

Table 2. *Residual proteolytic activity of human gastric proteinase 2 after preincubation with different triterpenoids (2g/l; 3.24mM) at pH7.4 and pH4.0 for 30min at 37°C*

Each value is expressed as a mean percentage of the control value.

Compound	Human gastric proteinase 2	
	pH7.4	pH4.0
Carbenoxolone [3-*O*-(β-carboxypropionyl)-11-oxo-18β-olean-12-en-30-oic acid]	14	41
1. 3-*O*-(γ-Carboxybutyryl)-11-oxo-18β-olean-12-en-30-oic acid	71	40
2. 11-Oxo-18β-olean-12-en-30-oic acid (3-*O*-sulphate disodium salt)	58	35
3. 3-*O*-(*cis*-2′-Carbonylcyclohexanecarboxylic acid)-11-oxo-18β-olean-12-en-30-oic acid (disodium salt)	41	7
4. 3-*O*-Lauroyl-11-oxo-18α-olean-12-en-30-oic acid	66	90
5. 3-Hydroxy-11-oxo-18β-olean-12-en-30-oic acid *N*-methylpiperazinamide (hydrochloride)	31	91
6. 3-Hydroxy-11-oxo-18β-olean-12-en-30-oic acid (glycinamide)	—	75
7. But-2-enyl-3-hydroxy-11-oxo-18β-olean-12-en-30-oate	63	90
8. 3-*O*,30-*O*-(Di-β-carboxypropionyl)olean-11,13(18)-diene (disodium salt)	60	33
9. Methyl 3,11-dioxo-18β-olean-12-en-30-oate (3-guanylhydrazone hydrochloride)	54	94
10. 3-*O*-(β-Carboxypropionyl)-11-oxo-18β-olean-12-en-30-oic acid (30-anthranilamide, disodium salt)	38	42
11. 3,4-seco-11-Oxo-18α-olean-4(23),12-diene-3,30-dioic acid (disodium salt)	66	42
12. Methyl 2,3-seco-11-oxo-18β-olean-12-ene-2,3-dioic acid 30-oate (disodium salt)	44	63
13. 3-*O*-(γ-Carboxybutyryl)-18β-olean-12-en-28-oic acid	40	25
14. 3-*O*-(β-Carboxypropionyl)-18β-olean-12-en-28-oic acid (disodium salt)	30	74
15. 3-*O*-(β-Carboxypropionyl)ursan-12-en-28-oic acid (disodium salt)	37	17
16. 3-*O*-(β-Carboxypropionyl)dihydrobetulinic acid (disodium salt)	32	23

Table 3. *Correlation coefficients for inhibition of human gastric proteinase 2 and the other gastric proteinases studied*

The correlation coefficients were calculated from the values given in Table 2 and the values given in Table 1 of Waft *et al.* (1974). The pH values indicate the pH of preincubation.

	Human gastric proteinase 2	
	pH7.4	pH4.0
Human pepsin 1	0.25	0.75 ($P<0.001$)
Human pepsin 3	0.09	0.67 ($0.01>P>0.001$)
Human pepsin 5	0.29	0.77 ($P<0.001$)
Pig pepsin A	0.33	0.61
Human gastric-mucosal extract	0.69 ($0.01>P>0.001$)	0.40
Human pepsinogen 1	0.93 ($P<0.001$)	0.27
Human pepsinogen 3	0.78 ($P<0.001$)	0.33
Human pepsinogen 5	0.68 ($0.01>P>0.001$)	0.11
Human gastric proteinase 2 (pH7.4)	—	0.19
Chymotrypsin	0.27	0.80 ($P<0.001$)
Trypsin	0.14	0.62 ($0.01>P>0.001$)

was found for the inhibition of pig pepsin (Kunimoto *et al.*, 1972).

Effect of carbenoxolone and related triterpenoids

The effect of carbenoxolone and related triterpenoids on the proteolytic activity of the proteinase was studied after preincubation of the enyzme with each compound at pH4.0 and at pH7.4. The residual proteolytic activity was then measured at pH3.7. Carbenoxolone inhibited the proteolytic activity markedly at both pH values as did compounds 3, 10, 13, 15 and 16 (Table 2). A comparison of the correlation coefficients between the inhibition of the proteinase at pH4.0 and at pH7.4 with the inhibition of other enzymes studied (Waft *et al.*, 1974) shows (Table 3) that the effect of the triterpenoid on proteinase activity at pH4.0 correlated significantly with the pepsins, whereas at pH7.4 significant correlation was shown with the pepsinogens and the gastric-mucosal extract. The inhibition of chymotrypsin and trypsin correlated significantly with that of the proteinases at pH4.0 but not at pH7.4. It should be noted that inhibition of the pepsins, but not the pepsinogens, correlated significantly with that of chymotrypsin and trypsin (Waft *et al.*, 1974).

Action on B-chain of oxidized insulin

Table 4 shows the sites of enzymic cleavage of the

Table 4. *Sites of action of human gastric proteinase 2 on the B-chain of oxidized insulin*

These were investigated after 5h incubation at pH1.7 and pH3.5; the specificity of human pepsins 3 and 5 (Etherington & Taylor, 1971; Roberts, 1975) and proteinase 1A (Etherington & Taylor, 1972) are also included for comparison. ←, Minor site of cleavage; ←, moderate site; ⇐, major site.

	Proteinase 2		Proteinase 1A		Human pepsin 3		Human pepsin 5	
	pH1.7	pH3.5	pH1.7	pH3.5	pH1.7	pH3.5	pH1.7	pH3.5
1. Phe								
2. Val				←		←		←
3. Asn								
4. Glu								
5. His					←	←		
6. Leu								
7. Cys(SO_3H)								
8. Gly								
9. Ser								
10. His								
11. Leu								
12. Val		←			←	←	←	←
13. Glu								
14. Ala		←		←	←	←	←	←
15. Leu				←	←	←	←	
16. Tyr	⇐	⇐			⇐	⇐	⇐	⇐
17. Leu	⇐	⇐		⇐	⇐	⇐	⇐	⇐
18. Val								
19. Cys(SO_3H)								
20. Gly								
21. Arg								
23. Gly								
24. Phe				←				←
25. Phe		←		⇐		←	⇐	⇐
26. Tyr	⇐	⇐		←	⇐	⇐	←	←
27. Thr								
28. Lys								
29. Pro								
30. Ala								

B-chain of oxidized insulin by proteinase 2 at pH1.7 and pH3.5 after 5h incubation. At pH1.7, after 24h, there was evidence of mild degradation of peptide 1–15 at bond 13–14 releasing the dipeptide Ala-Leu. This finding indicates that the $Leu_{(11)}$–$Val_{(12)}$ bond is not easily cleaved by proteinase 2 at pH1.7.

Degradation at pH3.5 was more extensive and three acidic residues of unknown composition were released after 24h incubation. Residues 1–11 and 25–30 were released at pH3.5 after 5h, indicating easier cleavage at the bonds $Leu_{(11)}$–$Val_{(12)}$ and $Phe_{(24)}$–$Phe_{(25)}$ at this pH than at pH1.7. The early release of peptide 25–30 suggests a primary site of cleavage, at the $Phe_{(24)}$–$Phe_{(25)}$ bond, but the relative amounts of this peptide were much less than of peptide 26–30, suggesting that the proteinase 2 has a higher affinity for the $Phe_{(25)}$–$Tyr_{(26)}$ bond.

The number of sites of action for proteinase 2 increased as the pH of incubation was raised and this paralleled the pH–activity curve for the enzyme on bovine haemoglobin, showing a maximum of activity between pH3.0 and pH3.7.

Discussion

The results show that a non-pepsin proteinase (proteinase 2) can be isolated from human gastric-mucosal extracts by the following steps: (*a*) chromatography on DEAE-cellulose at pH8.0 enabling the separation of non-pepsin from pepsin proteinase activity; (*b*) chromatography on CM-cellulose at pH5.5 enabling a separation of proteinase 2 from non-enzymic protein and from proteinase-1 components if present, as they are in extracts of gastric adenocarcinoma (Etherington & Taylor, 1972); (*c*) chromatography on DEAE-cellulose at pH5.3 to remove further non-enzymic protein; (*d*) repeat of (*a*) to separate the proteinase-2 components if possible. The product obtained in this way was not shown with certainty to contain two components,

but Etherington & Taylor (1972) found two components for the proteinase 2 of gastric adenocarcinomata.

During the examination of two stomachs by this procedure no evidence of the proteinase 1 was found, and it must be concluded, confirming Etherington & Taylor (1972), that the proteinases 1 do not occur in normal gastric mucosa but only in adenocarcinomata. Etherington & Taylor (1969) found the non-pepsin zone 7 to occur in the histamine-stimulated gastric juice of only 20% of normal subjects. It may be, therefore, that proteinase 2 is mainly an intracellular enzyme or that, after secretion, it is rapidly destroyed by the pepsins.

The pH–activity curve for the proteinase 2 from normal gastric mucosa was very similar to that of the proteinase 2 from gastric adenocarcinomata cells with a fairly extensive plateau-type maximum (c.f. Etherington & Taylor, 1972). The possibility exists that solutions of differing pH, between pH 1.5 and 5.5, contain different proportions of native and denatured haemoglobin and that such a difference affects the shape of the pH–activity curve. In practice this has been found not to happen (for references see Taylor, 1962) so that it has become the practice to determine pH–activity curves by the method used (Chiang *et al.*, 1969; Otto, 1971). Pepstatin inhibition characteristics for this enzyme were similar to those previously reported for both cathepsins D and E (Barrett & Dingle, 1972).

It is worth emphasizing that only small amounts of proteinase 2 occur in the gastric mucosa as compared with the much larger quantities of the pepsinogens or of the intracellular proteinases (cathepsins) in an organ such as the liver or spleen. This fact is germane to the earlier work of Willstatter & Bamann (1929), Freudenberg (1940), Buchs (1954) and other workers (see Taylor, 1959), who have described a gastric 'cathepsin' in relatively large amounts. Their evidence for this 'cathepsin' was derived from pH–activity curves, and it seems clear that they were working with either pepsin 5 ('gastricsin' of Richmond *et al.* 1958) or the second pH maximum near pH 3.5 of pepsin 3, the principal human pepsin (see Taylor, 1962). To avoid confusion we prefer the term proteinase for the non-pepsin enzymes of the present investigation.

Both proteinases 1 and 2 have similarities to the intracellular cathepsins of other tissues to which we should like to draw attention. Etherington & Taylor (1972) showed that the shape of the pH–activity curve of proteinase 1, its chromatographic and electrophoretic properties and its sites of action on B insulin were similar to those of the cathepsins D and also to human pepsin 5. The chromatographic and electrophoretic behaviour of the proteinase 2 was, they found, similar to that of cathepsin E from rabbit bone marrow (Lapresle & Webb, 1962). We have now shown that proteinase 2, in its sites of action on B insulin, differs from proteinase 1. Firstly, the $Leu_{(11)}$–$Val_{(12)}$ bond was readily cleaved by proteinase 2 at pH 3.5 but not by proteinase 1. Bohley *et al.* (1971) reported a cleavage of this bond by cathepsin E. The $Phe_{(25)}$–$Tyr_{(26)}$ bond was preferentially cleaved by proteinase 2, whereas proteinase 1 cleaved the $Phe_{(24)}$–$Phe_{(25)}$ bond (Etherington & Taylor, 1972).

Proteinase 2 could, therefore, well be considered to be a cathepsin E, but in its sites of action on B insulin it resembles also human pepsin 3 (Etherington & Taylor, 1971; Roberts, 1975). The importance of cathepsin E has been uncertain as it has been described only in the bone marrow. The presence of a cathepsin E in gastric mucosa and not a cathepsin D may perhaps indicate that tissues differ in their type of intracellular proteinase.

Waft *et al.* (1974) suggested from a correlation of the action of the triterpenoids on various proteolytic enzymes that specific groups on the triterpenoid molecule were important in the enzyme/inhibitor-binding process. The important groups thought to confer inhibitory activity were the free carboxy groups at C-30, C-28 and C-3 positions and possibly the chain length of the carboxy group at the C-3 position. The effect of pH on the correlation coefficients for proteinase 2 and the other proteinases studied appears to suggest, therefore, that the enzyme–triterpenoid binding is determined both by suitable chemical groups on the triterpenoid and by particular enzymic groups such that the triterpenoids bind effectively, but differently, both at pH 4.0 and at pH 7.4. What these enzymic groups or requirements are is not yet known but acylable groups, e.g. the hydroxy group of tyrosine and the lysine ε-amino group, may be likely candidates (Perlmann, 1966; Nakagawa & Perlmann, 1972). Thus proteinase 2 appears to possess structural characteristics that relate it to the pepsins and pepsinogens and biochemical properties that relate it to pepsin 3 and to cathepsin E.

References

Anson, M. L. & Mirsky, A. E. (1933) *J. Gen. Physiol.* **16**, 59–63

Barrett, A. J. & Dingle, J. T. (1972) *Biochem. J.* **127**, 439–441

Bohley, P., Kirschke, H., Langner, T., Ansorge, S., Wiederanders, B. & Hanson, H. (1971) in *Tissue Proteinases* (Barrett A. J. & Dingle, J. T., eds.), pp. 187–219, North-Holland, Amsterdam

Buchs, S. (1954) *Enzymologia* **16**, 193–201

Chiang, L., Sanchez-Chiang, L., Mills, J. N. & Tang, J. (1969) *J. Biol. Chem.* **242**, 3098–3102

Etherington, D. J. & Taylor, W. H. (1969) *Biochem. J.* **113**, 663–668

Etherington, D. J. & Taylor, W. H. (1970) *Biochem. J.* **118**, 587–594

Etherington, D. J. & Taylor, W. H. (1971) *Biochim. Biophys. Acta* **236**, 92–98
Etherington, D. J. & Taylor, W. H. (1972) *Clin. Sci.* **42**, 79–90
Freudenberg, E. (1940) *Enzymologia* **8**, 385–390
Ito, I., Guroff, G. & Udenfriend, S. (1964) *J. Biol. Chem.* **239**, 3385–3387
Kunimoto, S., Aoyagi, T., Morishima. H., Takeuchi, T. & Umezawa, H. (1972) *J. Antibiot.* **25**, 251–255
Lapresle, C. & Webb, T. (1962) *Biochem. J.* **84**, 455–462
Nakagawa, Y. & Perlmann, G. E. (1972) *Arch. Biochem. Biophys.* **49**, 476–482
Otto, K. (1971) in *Tissue Proteinases* (Barrett, A. J. & Dingle, J. T., eds.), pp. 1–28, North-Holland, Amsterdam
Perlmann, G. E. (1966) *J. Biol. Chem.* **241**, 153–157
Richmond, V., Tang, J., Trucco, R. E. & Caputto, R. (1958) *Biochim. Biophys. Acta* **29**, 453–454
Roberts, N. B. (1975) Ph.D. Thesis, University of Liverpool
Roberts, N. B. & Taylor, W. H. (1973) *Clin. Sci. Mol. Med.* **45**, 213–224
Sanger, F. (1949) *Biochem. J.* **44**, 126–128
Sanger, F. & Tuppy, H. (1951) *Biochem. J.* **49**, 481–490
Taylor, W. H. (1959) *Biochem. J.* **71**, 73–83
Taylor, W. H. (1962) *Physiol. Rev.* **42**, 519–553
Waft, L. A., Roberts, N. B. & Taylor, W. H. (1974) *Clin. Sci. Mol. Med.* **46**, 519–526
Willstatter, R. & Bamann, E. (1929) *Hoppe-Seyler's Z. Physiol. Chem.* **180**, 127–140